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# Anti-Diabetes Activity Of Acacia farnesiana (L.) Willd In Alloxan Diabetic Rats

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**Abstract:** The aim of the study was to scientifically validate traditional anti-diabetic claim of *Acacia farnesiana* and to determine its utility as a potential therapeutic agent. An active fraction from aqueous extract was obtained by alcohol precipitation method and anti-hyperglycemic activity was evaluated in normal glucose loaded rats. Anti-diabetes activity of the fraction was evaluated in alloxan-induced diabetic rats and short term toxicity evaluation was done in adult male mice. The active fraction showed promising anti-diabetic activity at a dose of 25 mg/kg. The active fraction was devoid of any conspicuous toxic symptoms even at 400 mg/kg. The active fraction isolated from *Acacia farnesiana* is an attractive material for further studies leading to the development of an anti-diabetes phyto-medicine or conventional pure chemical entity medicine.
**Keywords:** *Acacia farnesiana* (L.); Anti-diabetes; Alloxan induced diabetic rats; Anti-hyperglycemic activity.

## INTRODUCTION

Diabetes Mellitus (DM) is a major degenerative disease [1,2] affecting at least 10% of the population, worldwide. Complications of DM include hypertension, atherosclerosis, microcirculatory disorders, retinopathy, nephropathy, neuropathy and angiopathy [3]. Despite the availability of several antidiabetic drugs, DM is still uncurable. It is a lifelong treatment in almost all of the cases and the patients may develop toxicity and/or insensitivity to the drugs with their prolonged use. It has been well documented that only a small proportion of the diabetes patients get opportunity for modern therapy due to poor socio economic status. Hence, a larger percentage of the population depends on herbal drugs to control DM.

To date more than 100 plants in India exhibit varying levels of hypoglycemic activity [4]. Several diabetic herbal formulations are also available in Indian Market as traditional medicine [5]. However, herbal drugs with the promising anti-diabetic effects are only a few.

*Acacia farnesiana* is a medicinal plant that grows throughout tropical parts of Indian subcontinent, particularly in sandy soils of river beds in Northern India and parts of Tamil Nadu. It is used in folk medical practices to treat DM in certain remote villages of Thirunelveli, district Tamil Nadu. In ethno-medical practices, the plant is also used as

diuretic, treat antiulcer, anti-pyritic etc. Absence of evidence on anti-diabetic activity of *Acacia farnesiana* let us embark on this study with an aim to scientifically prove the traditional claim of this plant.

## MATERIALS AND METHODS

### Collection of plant materials

Aerial parts of *Acacia farnesiana* were collected from Tirunelveli district of Tamil Nadu and identified by Dr. Mathew Don, Taxonomist of Tropical Botanic Garden and Research Institute (TBGRI) and a voucher specimen, TBGRI 8283, was deposited in the herbarium of TBGRI.

### Chemicals

Alloxan was purchased from LOBA Chemie Pvt Ltd. Mumbai; All other chemicals used were of analytical grade and purchased from E. Merck Ltd., Mumbai.

### Preparation of *Acacia farnesiana* extracts

The aerial parts of the plant were collected, cleaned, dried and powdered. The aqueous suspension of the aerial part was prepared by grinding the powder in 2% gum acacia (w/v). A 10 % suspension of *Acacia farnesiana* (aerial parts) was used in the initial screening using glucose tolerance test (GTT).

To prepare water extract, the powder was extracted with distilled water (5 g/100 ml) by stirring for 4 hours and then filtering through filter paper (Watman No.1). This process was repeated thrice with the residue. The combined filtrate was freeze-dried in a lyophilizer.

The alcoholic and n-hexane extract of the aerial part of the plant powder was prepared similarly using ethyl alcohol and n-hexane respectively. The process was repeated three times. The alcohol and hexane extracts were dried using a rotary evaporator under reduced pressure at 40$^{o}$C.

### Isolation of an active fraction

The water extract of the plant was precipitated with ethyl alcohol (1:1) and separated into precipitate fraction and alcohol soluble fraction; both of the fractions were tested for anti-hyperglycemic activity using GTT. The active fraction was subjected to phyto-chemical analysis to determine the presence of compounds [6].

### Animals

Inbred Wistar rats (150-200 g weight) reared in TBGRI animal house were used for in vivo experimentation. Animals were caged in uniform hygienic conditions and fed with standard pellet diet (Lipton Indian Ltd, Bangalore) and water *ad libitum* as per the guidelines of Institute Animal Ethics Committee (IAEC). IAEC is approved by CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals).

### Glucose tolerance test (GTT)

GTT was used to investigate the glucose lowering effect of the extracts and active fraction. For this, normal rats were divided into two groups. Control group received the vehicle (2% gum acacia, 1 ml/ rat, p.o.). The experimental groups received the extract as indicated in an identical manner. In the initial screening for activity, a relative high dose (500 mg/kg) of the water suspension of the plant powder in 2% gum acacia was used]. The rats of all the groups were loaded with 60% glucose (3 g/kg, p.o.), 30 min after herbal drug or vehicle (control) administration. Blood glucose estimation is done by one touch electronic glucometer (Horizon) using glucose strips just 1min prior to extract administration, and at 30 and 90 min after glucose loading. Six fed animals were used in each group.

Similarly, the different extracts, different doses of the active water extract in fed and fasted rats and fractions of water extract in fed rats were subjected to GTT.

### Alloxan-induced diabetic rats

As described elsewhere [7], male Wistar rats (190-210 g body weight) were injected with alloxan (110 mg/kg) through intraperitonial route. Five days later, blood samples were drawn and glucose levels were determined to confirm induction of diabetes. The diabetic rats which showed blood glucose levels in the range of 19.0 – 24.0 mmol/L were selected for efficacy evaluation of the herbal drug.

### Determination of the efficacy of the active fraction in alloxan diabetic rats

The alloxan diabetic rats were divided into three groups of six each. The diabetic control rats were given 1.5 ml of water, p.o., daily. The test group was given daily dose of active fraction (25 mg/kg). While the third group (positive control group) received glibenclamide (5mg/kg.). Weight and sex matched six normal rats were kept as control group (Normal control group). Treatment was continued for 12 days. Blood samples were collected 1h after administration of the herbal drug or insulin on days 1, 4, 8 and 12. On day 12, animals were sacrificed after blood collection and liver samples were removed for glycogen estimation.

### Estimation of liver glycogen

Liver glycogen was estimated by the method of Carrol et al [8].

**Toxicity evaluation in mice**

To evaluate sub-acute (short term) toxicity, four groups of mice, each containing six male mice (20-25 g body weight) were used. Group one group was kept as control and groups 2, 3, and 4 received 100, 200, and 400 mg/kg of active fraction, respectively, for 28 days (p.o.). Control group received the vehicle in an identical manner.

The behavior of the animals was observed daily for 1h for 28 days. Initial and final body weights, water and food intake, state of stool and body temperature were also observed. The animals were sacrificed on the 28th day. Hemoglobin was measured using hemoglobinometer with comparison standards. Glutamate pyruvate transaminase (GPT) and Glutamate oxaloacetate transaminase (GOT) [9] and alkaline phosphatase [10] were measured following standard methods. Urea, cholesterol, total lipids and protein were determined by conventional methods [11]. Important organs were dissected out, weighed and observed for pathological and morphological changes.

**Statistical analysis**

Statistical comparison was done using one- way ANOVA followed by Dunnets post hoc comparison when more than two groups were involved. P values less than 0.05 were considered significant. When the number of groups was 2, student's t-test was used to test the level of significance.

## RESULTS

The crude water suspension of *Acacia farnesiana* (500 mg/kg) showed significant reduction in the blood glucose levels in fed and glucose loaded (GTT) rats at 30 and 90 min after glucose administration and the percentage reduction was more at 30 minutes compared to that at 90 minutes (Table 1).

When different extracts of the plant were tested for their glucose lowering effects, the water extract at a dose of 50 mg/kg showed significant glucose lowering activity at 30 and 90 minutes after glucose loading in normal fed rats. The alcohol and hexane extracts of these plants were almost inactive (Table 2).As given in table 3, the extract (50 mg/kg) was more effective in lowering the blood glucose levels in fed rats, not in the fasted rats. The water extract showed optimum activity at 50 mg/kg and at a higher dose (100 mg/kg) it did not show a proportional increase in activity. However, even at higher doses, hypoglycemic effects were not observed. The yield of precipitate and soluble fractions of the water extract obtained by alcohol precipitation was approximately 48 and 52%, respectively. The glucose lowering effects of the fractions are given in Table 4. The soluble fraction showed significant glucose lowering activity in normal glucose loaded rats at a dose of 25 mg/kg. The precipitate fraction was devoid of the activity. As given in Table 5, the active fraction (soluble fraction) showed significant anti-diabetes activity in alloxan diabetic rats. The daily drug administration at a dose of 25 mg/kg gradually decreased the blood glucose levels from 19.7 to12.8 mmol/L in 12 days. Glibenclamide (5 mg/kg) was found to have almost the same effects of the herbal drug.The herbal drug also restored the body weight, liver weight and liver glycogen values to normal levels. In untreated diabetic control rats, the glucose levels increased from 19.4 to 23.4 mmol/L during the same period. The body weight loss and drastic reduction in liver glycogen, observed in the diabetic animals were prevented to a large extent by the drug administration and the effect of the herbal drug on these parameters is almost comparable to that of Glibenclamide (Table 6).In sub-acute toxicity evaluation the active fraction did not show any significant effect on behavior as well as serum biochemical parameters and hematological parameters (Table 7 and 8).

**Table 1: Effect of water suspension of *A.farnesiana* (aerial parts) on glucose tolerance in fed and glucose loaded normal rats**

| Treatment | Serum glucose levels (mmol/l) | | |
|---|---|---|---|
| | 0 minutes* | 30 minutes* | 90 minutes* |
| **Control (2% gum acacia)** | 3.07 ± 0.18 | 6.38 ± 0.33 | 3.95 ± 0.23 |
| **Aerial powder, water suspension (500 mg/kg)** | 3.00 ± 0.17 | 4.74± 0.23*** | 3.86 ± 0.21 |

*Values are mean ±SD; n=6; *Student t-test ** P<0.01, *** P<0.001 (compared to respective control values);0 time, initial glucose level just before drug administration; 30 min and 90 min, time after glucose loading*

**Table 2: Effect of different extracts of *A.farnesiana* (aerial part) on glucose tolerance in fed and glucose loaded normal rats**

| Treatment | Serum glucose levels (mmol/l) | | |
|---|---|---|---|
| | 0 minutes*(initial) | 30 minutes* | 90 minutes* |
| **Control** | 5.13± 0.30 | 9.85± 0.43 | 7.68± 0.23 |
| **Water extract (50 mg/kg)** | 5.19± 0.27 | 6.55± 0.29*** | 6.94± 0.19** |
| **Alcohol extract (50 mg/kg)** | 5.23± 0.26 | 9.41± 0.48 | 7.76± 0.44 |
| **Hexane extract (50 mg/kg)** | 5.39 ± 0.33 | 9.35± 0.60 | 7.80± 0.36 |

*Values are mean ±S.D.; n=6; *Student t-test ***, P<0.001, **, P<0.05 (compared to control values, Student's t test)*

**Table 3: Effect of different doses of *A.farnesiana* (aerial part) on glucose tolerance in fed and fasted glucose loaded normal rats**

| Treatment | Serum glucose levels (mmol/l) | | |
|---|---|---|---|
| | 0 minutes* | 30 minutes* | 90 minutes* |
| | **Fed rats** | | |
| **Control** | 5.05 ± 0.25 | 9.81 ± 0.49 | 7.19 ± 0.30 |
| **Water extract (25 mg/kg )** | 5.17 ± 0.20 | 7.30 ± 0.25*** | 6.78 ± 0.29 |
| **Water extract (50 mg/kg)** | 5.15 ± 0.26 | 6.26 ± 0.32*** | 6.39 ± 0.24** |
| **Water extract (100 mg/kg)** | 5.24 ± 0.30 | 5.83 ± 0.33*** | 6.20 ± 0.22** |
| | **Fasted rats** | | |
| **Control** | 2.93 ± 0.15 | 6.50 ± 0.33 | 4.57 ± 0.21 |
| **Water extract (25 mg/kg)** | 3.08 ± 0.19 | 6.16 ± 0.25 | 4.39 ± 0.31 |
| **Water extract (50 mg/kg)** | 2.87 ± 0.19 | 6.04 ± 0.32 | 4.40 ± 0.25 |
| **Water extract (100 mg/kg)** | 2.97 ± 0.25 | 6.10 ± 0.28 | 4.22 ± 0.28 |

*Values are mean ±S.D.; n=6; *Student t-test ** P<0.01, *** P<0.001 (compared to control values); 0 min: initial glucose level just before drug administration; 30 minutes and 90 minutes: time after glucose loading*

**Table 4: Effect of the two fractions of the aqueous extract of *A.farnesiana* on glucose tolerance in fed and glucose loaded normal rats**

| Treatment | Serum glucose levels (mmol/l) | | |
|---|---|---|---|
| | 0 minutes* (initial) | 30 minutes* | 90 minutes* |
| **Control** | 4.94 ± 0.30 | 9.57 ± 0.50 | 7.09 ± 0.29 |
| **Water extract (50 mg/kg)** | 5.07 ± 0.26 | 6.16 ± 0.05*** | 5.40 ± 0.24** |
| **Precipitate fraction (25 mg/kg)** | 4.95 ± 0.34 | 9.31 ± 0.40 | 7.17 ± 0.29 |
| **Soluble fraction (25 mg/kg)** | 5.12 ± 0.24 | 6.03 ± 0.37*** | 5.45 ± 0.26** |

*Values are mean ±S.D., n=6; *Student t-test ** P<0.01, *** P<0.001 (Compared to control)*

**Table 5**: **Effect of the active fraction of *A.farnesiana* on blood glucose level in alloxan-induced diabetic rats**

| Groups | Serum glucose levels (mmol/l) | | | |
|---|---|---|---|---|
| | 1st day* | 4th day* | 8th day* | 12th day* |
| **Normal control rats** | 5.2 ± 0.20 | 5.5 ± 0.16 | 5.7 ± 0.10 | 5.4 ± 0.08 |
| **Diabetic control rats** | 19.4 ± 0.29 | 21.1 ± 0.28 | 22.3 ± 0.20 | 23.4 ± 0.17 |
| **Diabetic rats treated with active fraction (25 mg/kg)** | 19.7 ± 0.35 | 18.6 ± 0.30** | 15.9 ± 0.12** | 12.8 ± 0.19*** |
| **Diabetic rats treated with Glibenclamide (5 mg/kg)** | 20.1 ± 0.31 | 18.0 ± 0.30** | 15.3 ± 0.29** | 12. 0 ± 0.80*** |

*Values are mean ±S.D., n=6, *Student t-test **P<0.05, *** P<0.001, (Compared to diabetic control)*

**Table 6**: **Effect of the active fraction on liver weight and liver glycogen in alloxan-induced diabetic rats**

| Treatment | Body weight (g) | | Liver weight (g) | Liver glycogen (mg/g of the wet tissue) |
|---|---|---|---|---|
| | Initial day | Final day (12th day) | | |
| **Normal control** | 204.3 ± 8.5 | 227.3 ± 7.8 | 4.94 ± 0.95 | 11.98 ± 1.46 |
| **Diabetic control** | 202.2 ± 10.2 | 189.3 ± 9.1** | 3.70 ± 0.18** | 7.19 ± 0.13*** |
| **Diabetic treated with active fraction (25 mg/kg)** | 200.0 ± 9.2 | 226.6 ± 7.1 | 4.60 ± 0.12 | 10.74 ± 0.15 |
| **Diabetic treated with Glibenclamide (5mg/kg)** | 205.0 ± 11.1 | 222.5 ± 10.1 | 4.83 ± 0.22 | 11.09 ± 0.22 |

*Values are mean ±S.D: n=6; *Student t-test ** P<0.05, *** P<0.001 (Compared to normal control values)*

**Table 7**: **Effect of the active fraction of *A.farnesiana* on serum biochemical parameters in short term (28 days) toxicity studies**

| Parameters | Active fraction | | | |
|---|---|---|---|---|
| | Control (0) | 100 | 200 | 400 |
| SGPT (IU/L) | 10.3 ± 0.9 | 10.0 ± 1.2 | 11.0 ± 1.0 | 12.0 ± 2.0 |
| SGOT (IU/L) | 18.7 ± 1.7 | 18.3 ± 0.7 | 18.5 ±1.5 | 19.0 ± 1.9 |
| ALP (IU/L) | 67.6 ± 2.8 | 70.0 ± 2.1 | 66.5 ± 2.5 | 70.0 ± 2.3 |
| Urea (mmol/L) | 9.1 ± 0.9 | 9.2 ± 0.8 | 9.4 ± 1.0 | 9..3 ± 1.1 |
| Glucose (mmol/L) | 5.6 ± 0.3 | 5.4 ± 0.3 | 5.1 ± 0.3* | 5.0± 0.3** |
| Cholesterol (mmol/L) | 4.1 ± 0.2 | 4.1 ± 0.3 | 4.2.0± 0.2 | 4.1 ± 0.3 |
| HDL (mg/dl) | 47.6 ± 1.9 | 50.0 ± 1.2 | 48.5 ± 2.5 | 50.0 ± 2.3 |
| LDL (mg/dl) | 97.1 ± 0.9 | 96.1 ± 0.6 | 96.1 ± 1.0 | 96.0 ± 0.4 |
| Triglycerides (mmol/dl) | 6.1 ± 0.2 | 6.2 ± 0.3 | 6.1 ± 0.3 | 6.3 ± 0.2 |
| Creatinine (µmol/L) | 88.3 ± 4.1 | 85.3 ± 5.1 | 91.1 ± 5.3 | 93.3 ± 5.9 |
| Total protein ( g/L) | 73.2 ± 1.9 | 72.1 ± 2.1 | 74.0 ± 2.5 | 69. 5 ± 4.3 |
| Albumin (g/L) | 47.3 ± 4.4 | 46.4 ± 2.4 | 48. 8 ± 3.4 | 49.5 ± 5.1 |
| Globulin (g/L) | 35.0 ± 2.2 | 36.1 ± 2.3 | 37.0 ± 2.2 | 37.1 ± 5.0 |
| Direct bilurubin (µmol/L) | 2.22 ± 0.13 | 2.21 ± 0.14 | 2.30 ± 0.15 | 2.32 ± 0.13 |
| In direct bilurubin (µmol/L) | 3.26 ± 0.23 | 3.23 ± 0.25 | 3.27 ± 0.25 | 3.76 ± 0.22* |

*Values are mean ±S.D ; n=6, * P<0.05, ** P<0.01 (compared to control); SGPT, serum glutamate pyruvate transaminase; SGOT, serum glutamate oxalate transaminase; ALP, alkaline phosphatase; LDL, low density lipoprotein; HDL, high density lipoprotein*

**Table 8: Effect of the active fraction of *A.farnesiana* on hematological parameters in short term (28 days) toxicity studies**

| | Active fraction (mg/kg) | | | |
|---|---|---|---|---|
| **Parameters** | **Control (0)** | **100** | **200** | **400** |
| Hb (gm%) | 14.73 ± 0.4 | 14.60 ± 0.2 | 14.80 ± 0.3 | 14.90 ± 0.5 |
| WBC ($mm^3$) | 6900 ± 278 | 6925 ± 325 | 6975 ± 425 | 6100 ± 50 |
| RBC(x $10^{-6}/mm^3$) | 8.75 ± 0.15 | 8.76 ± 0.14 | 8.85 ± 0.10 | 8.00 ± 0.10 |
| PCV (%) | 45.00 ± 1.30 | 45.80 ± 1.50 | 45.95 ± 1.65 | 45.50 ± 0.95 |
| MCV (fl) | 92.59 ± 5.41 | 94.35 ± 4.65 | 94.75 ± 4.42 | 94.15 ± 1.35 |
| MCHC (g/dl) | 34.00 ± 4.50 | 36.36 ± 2.78 | 36.61 ± 1.31 | 37.80 ± 0.78 |
| **Differential count** | | | | |
| Neutrophils (%) | 42.33 ± 0.88 | 42.00 ± 0.57 | 43.00 ± 1.00 | 43.33 ± 1.40 |
| Lymphocytes (%) | 66.33 ± 1.73 | 67.66 ± 1.45 | 65.70 ± 2.00 | 67.50 ± 1.45 |
| Eosinophils (%) | 4.66 ± 0.88 | 4.33 ± 2.18 | 4.50 ± 1.50 | 4.93 ± 1.33 |
| Monocytes (%) | 4.73 ± 0.43 | 4.60 ± 0.25 | 4.80 ± 0.44 | 4.90 ± 0.50 |

*Values are mean ±S.D.; n=6 (compared to control, values are not significant); PCV, Packed cell volume; MCV, Mean corpuscular volume; MCHC, Mean corpuscular hemoglobin concentration*

## DISCUSSION

Existing literature fails to report pharmacological and phyto-chemical studies of *Acacia farnesiana*. Therefore the present study was under taken to test the anti-hyperglycemic activity of *Acacia farnesiana* (aerial parts) in rats with the aim to verify its traditional claim as an anti-diabetic agent. The active fraction obtained from this plant is an attractive material for further studies leading to drug development. Development of phyto-medicines is relatively inexpensive and less time consuming. This is more suitable for low and middle income countries. However, ecotype, genotype and seasonal variations in efficacy and safety, if any, have to be determined in phyto-medicine development.

Unlike insulin, the herbal drug does not possess severe hypoglycemic effect in rats. The water extract showed optimum activity at 50 mg/kg and a further increase to 100 mg/kg did not result in a significant decrease in glucose levels. Thus, it appears that unlike insulin and sulfonylureas [12], over dose of this herbal drug may not result in hypoglycemia. Further, 25 mg/kg active fraction showed activity comparable to that of 5 mg/kg Glibenclamide.

In the present study, severe alloxan diabetic model was used where the initial blood glucose level was more than 19.4 mmol/L. This model is almost comparable to type one diabetes model with near total β cell destruction and insulin resistance [13]. Studies are in progress to elucidate the mechanism of action of this herbal drug.

When the active fraction was evaluated for its toxicity, it did not exhibit any conspicuous toxic symptoms in the short term toxicity evaluation in mice.

The plant is promising for further studies leading to the development of a valuable medicine for diabetes.

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